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DNA-PK-dependent binding of DNA ends to plasmids containing nuclear matrix attachment region DNA sequences: evidence for assembly of a repair complex.

Mauldin SK, Getts RC, Liu W, Stamato TD.

Lankenau Institute for Medical Research, 100 Lancaster Avenue, Wynnewood, PA 19096, USA and Genisphere, Incorporated, 4170 City Avenue, Philadelphia, PA 19131-1694, USA.

We find that nuclear protein extracts from mammalian cells contain an activity that allows DNA ends to associate with circular pUC18 plasmid DNA. This activity requires the catalytic subunit of DNA-PK (DNA-PKcs) and Ku since it was not observed in mutants lacking Ku or DNA-PKcs but was observed when purified Ku/DNA-PKcs was added to these mutant extracts. Purified Ku/DNA-PKcs alone did not produce association of DNA ends with plasmid DNA suggesting that additional factors in the nuclear extract are necessary for this activity. Competition experiments between pUC18 and pUC18 plasmids containing various nuclear matrix attachment region (MAR) sequences suggest that DNA ends preferentially associate with plasmids containing MAR DNA sequences. At a 1:5 mass ratio of MAR to pUC18, approximately equal amounts of DNA end binding to the two plasmids were observed, while at a 1:1 ratio no pUC18 end binding was observed. Calculation of relative binding activities indicates that DNA end-binding activities to MAR sequences was 7-21-fold higher than pUC18. Western analysis of proteins bound to pUC18 and MAR plasmids indicates that XRCC4, DNA ligase IV and scaffold attachment factor A preferentially associate with the MAR plasmid in the absence or presence of DNA ends. In contrast, Ku and DNA-PKcs were found on the MAR plasmid only in the presence of DNA ends suggesting that binding of these proteins to DNA ends is necessary for their association with MAR DNA. The ability of DNA-PKcs/Ku to direct DNA ends to MAR and pUC18 plasmid DNA is a new activity for DNA-PK and may be important for its function in double-strand break repair. A model for DNA repair based on these observations is presented.

PMID: 12235392 [PubMed - in process]

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Tying loose ends: roles of Ku and DNA-dependent protein kinase in the repair of double-strand breaks.

Lieber MR, Grawunder U, Wu X, Yaneva M.

Division of Molecular Oncology, Departments of Pathology, Biochemistry and Molecular Biophysics, Campus Box 8118, Washington University Schoolof Medicine, 660 South Euclid Avenue, St. Louis, Missouri 63110, USA. lieber@pathology.wustl.edu

A convergence of information from biochemistry, yeast and mammalian genetics, immunology, and radiation biology has permitted identification of some of the protein participants - Ku, DNA-PK, XRCC4 - and the reaction intermediates in DNA end joining, suggesting how broken chromosomal ends may be recognized and repaired in eukaryotic cells. Some components may be defective in inherited disorders.

Publication Types:
- Review
- Review, Tutorial

PMID: 9024627 [PubMed - indexed for MEDLINE]

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